

NOVEL POLYPEPTIDE

Technical field

Jac The present invention relates to a novel polynucleotide sequence which encodes a novel polypeptide belonging to the class of proteins known as G-protein coupled receptors (GPCRs). The present invention also relates, inter alia, to processes for producing the polypeptide and its uses.

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Background of the invention

Cells and tissues respond to a wide variety of extracellular signalling molecules through the interaction of these molecules with specific cell-surface receptors. One such class of receptors are known as G-protein coupled receptors (GPCRs) and these are characterised by containing a series of 7 hydrophobic transmembrane segments. Upon binding of an extracellular ligand to its receptor, intracellular signals are initiated via interactions with heterotrimeric G proteins which in turn can lead to a number of different intracellular events depending upon which receptor has been activated. For example some GPCRs influence adenyl cyclase activity whereas others act via phospholipase C.

Members of the GPCR superfamily respond to a wide variety of ligands including small molecule amines (such as serotonin, dopamine, acetylcholine), lipid-derived mediators (such as LpA), amino acid derivatives (such as glutamate) and neurotransmitter peptides and hormones (such as neurokinin, galanin, glucagon, gastrin). Although GPCRs are activated by a broad range of ligands, it should be noted that individual GPCRs have a small and very specific repertoire of ligands. Based upon an analysis of the primary structure of a novel GPCR, it is now possible to classify them into specific sub-families, thereby narrowing the range of potential ligands.

In many cases, the endogenous ligands of GPCRs are relatively small, enabling them to be mimicked or blocked by synthetic analogues. For example drugs such as prazosin,

doxazosin, cimetidine, ranitidine are all effective antagonists of their respective target GPCRs.

Thus, as the activation or inhibition of GPCRs can have therapeutic consequences, there
5 is a continued need to provide new GPCRs and their associated agonists and antagonists.

Summary of the invention

10 According to one aspect of the present invention, there is provided an isolated polynucleotide comprising:

- (a) a polynucleotide encoding the polypeptide as set forth in SEQ ID NO: 2;
 - (b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;
 - 15 (c) a polynucleotide comprising a nucleotide sequence that has at least 70% identity, preferably at least 70-75% identity; to the polynucleotide of (a) or (b);
 - (d) a polynucleotide comprising a nucleotide sequence which is capable of hybridising to a polynucleotide according to any one of (a) to (c); or
 - (e) a polynucleotide fragment of a polynucleotide according to any one of (a)
- 20 to (d).

According to other aspects of the present invention, there is provided an isolated polynucleotide essentially consisting of, or an isolated polynucleotide consisting of, a polynucleotide according to any one of (a) to (e) above.

25 Preferably, the polynucleotide comprises a nucleotide sequence that has at least 75% identity, preferably at least 75-80% identity to a polynucleotide according to (a) or (b) above. More preferably, the polynucleotide comprises a nucleotide sequence that has at least 80% identity, preferably at least 80-85% identity to a polynucleotide according to
30 (a) or (b) above. Even more preferably, the polynucleotide comprises a nucleotide sequence that has at least 85% identity, preferably at least 85-90% identity to a polynucleotide according to (a) or (b) above. More preferably, the polynucleotide comprises a nucleotide sequence that has at least 90% identity, preferably at least 90-95% identity to a polynucleotide according to (a) or (b) above. Yet more preferably, the

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polynucleotide comprises a nucleotide sequence that has greater than 95% identity to a polynucleotide according to (a) or (b) above. Preferably, the polynucleotide comprises a nucleotide sequence that has at least 98% identity to a polynucleotide according to (a) or (b) above.

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According to another aspect of the present invention, there is provided a polypeptide comprising:

- 10 (a) a polypeptide having the deduced amino acid sequence translated from the polynucleotide sequence in SEQ ID NO: 1, and variants, fragments, homologues, analogues and derivatives thereof; or
- (b) a polypeptide of SEQ ID NO: 2, and variants, fragments, homologues, analogues and derivatives thereof.

15 In a preferred embodiment, the polypeptide consists of SEQ ID NO: 2.

In another preferred embodiment, the polypeptide consists of a variant, fragment, homologue, analogue or derivative of SEQ ID NO: 2.

20 In a preferred embodiment, a polynucleotide according to the invention encodes a G-protein coupled receptor (GPCR).

The present invention also provides a polynucleotide probe or primer comprising at least 15 contiguous nucleotides of a polynucleotide according to the invention.

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The present invention also provides a recombinant DNA comprising a polynucleotide sequence according to the invention.

30 The present invention yet further provides a vector comprising a polynucleotide according to the invention.

According to a further aspect of the present invention, there is provided a host cell comprising a vector according to the invention, preferably a host cell transformed or

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transfected with a vector according to the invention. Preferably, the host cell is a mammalian, insect, bacterial, fungal or yeast cell.

According to yet a further aspect of the present invention, there is provided a process for
5 producing a polypeptide according to the invention, or fragment thereof comprising
culturing said host cell comprising the vector under conditions sufficient for the
expression of said polypeptide or fragment. Preferably, said polypeptide or fragment is
expressed at the surface of said cell. The process preferably further includes recovering
the polypeptide or fragment from the culture. There is also provided a membrane
10 preparation of said cells.

The present invention provides a process for producing cells capable of expressing a polypeptide or fragment thereof comprising transforming or transfecting cells with a vector according to the invention.

15 According to a further embodiment of the present invention, there are provided cells produced by the process of transformation or transfection with a vector according to the invention. There is also provided a membrane preparation of said cells.

20 There is also provided by the present invention an antibody against a polypeptide according to the invention.

The present invention yet further provides a compound which activates a polypeptide according to the invention (an agonist), or a compound which inhibits activation of a
25 polypeptide according to the invention (an antagonist).

According to another aspect of the present invention, there is provided a method for identifying an agonist compound which can bind to and activate a polypeptide according to the invention comprising:

30 (a) contacting the compound to be tested with cells expressing on the surface thereof a polypeptide according to the invention or a membrane preparation of said cells, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of an agonist compound

to said polypeptide; said contacting being under conditions sufficient to permit binding of agonist compounds to the polypeptide; and

- 5 (b) identifying an agonist compound capable of polypeptide binding and activation by detecting the signal produced by said second component.

According to another aspect of the present invention, there is provided a method for identifying an antagonist compound which can bind to and inhibit activation of a polypeptide according to the invention comprising:

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- (a) contacting (i) a detectable first component known to bind to and activate a polypeptide according to the invention and (ii) the compound to be tested with cells expressing on the surface thereof polypeptide according to the invention or a membrane preparation of said cells, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of the detectable first component to said polypeptide; said contacting being under conditions sufficient to permit binding of the detectable first component or agonist compounds to the polypeptide; and
- (b) determining the extent to which the binding of the first component to the polypeptide is affected by the test compound, by detecting the absence or otherwise of a signal generated from the interaction of the first component with the polypeptide.

As GPCRs are involved in signal transduction, agonists or antagonists of the polypeptide of the present invention can find use in interfering in, that is modulating the signal transduction process. Consequently, the present invention provides an agonist compound which can activate a GPCR polypeptide according to the invention, or an antagonist compound which can inhibit activation of a GPCR polypeptide according to the invention for use as a pharmaceutical. Agonists or antagonists of the GPCR polypeptide, can find use in therapeutic areas in which conditions and diseases are affected by signal transduction. For example, agonists or antagonists of the polypeptide, can find use in therapeutic areas such as: inflammation, allergy and respiratory, neurology, psychotherapy, urogenital disease, reproductive and sexual dysfunction/disorders, cancer,

tissue repair, dermatology, skin pigmentation disorders, photoageing, frailty, osteoporosis, metabolic disease, cardiovascular disease, gastrointestinal disease, anti-infection, sensory organ disorders, sleep disorders and hairloss.

- 5 Accordingly, there is also provided the use of an agonist of a GPCR polypeptide according to the invention in the manufacture of a medicament for the treatment of a patient having a condition or disease which can be ameliorated by activation of the GPCR polypeptide.
- 10 There is also provided the use of an antagonist of a GPCR polypeptide according to the invention, in the manufacture of a medicament for the treatment of a patient having a disease or condition which can be ameliorated by inhibition of the GPCR polypeptide.

According to yet a further aspect of the invention, there is provided a method for the treatment of a patient having a disease or condition which can be ameliorated by activation of a GPCR polypeptide according to the invention, comprising administering to the patient a therapeutically effective amount of an agonist compound according to the invention. Preferably, said agonist compound is a polypeptide and a therapeutically effective amount of said polypeptide is provided by administering to the patient DNA 20 encoding said polypeptide and expressing said polypeptide in vivo.

According to yet a further aspect of the invention, there is also provided a method for the treatment of a patient having a disease or condition that can be ameliorated by inhibition of a GPCR polypeptide according to the invention, comprising administering to the patient a therapeutically effective amount of an antagonist compound according to the invention. Preferably, said antagonist compound is a polypeptide and a therapeutically effective amount of said polypeptide is provided by administering to the patient DNA 25 encoding said polypeptide and expressing said polypeptide in vivo.

- 30 There is also provided by the present invention a method for the treatment of a patient having need, depending on the condition or disease to be treated, either to activate or to inhibit a GPCR polypeptide according to the invention, comprising administering to the patient a therapeutically effective amount of an antibody according to the invention.

Yet further provided by the present invention is use of an antibody according to the invention in the manufacture of a medicament for the treatment of a patient having need, depending on the condition or disease to be treated, either to activate or to inhibit a GPCR polypeptide according to the invention.

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According to a further aspect of the present invention, there is provided a method of treatment of a patient having need to upregulate a GPCR polypeptide according to the invention, comprising administering to the patient a therapeutically effective amount of a polypeptide according to the present invention. Preferably, said therapeutically effective 10 amount of the polypeptide is provided by administering to the patient DNA encoding said polypeptide and expressing said polypeptide in vivo.

There is also provided by the present invention, use of a polypeptide according to the invention in the manufacture of a medicament for the treatment of a patient having need 15 to upregulate a GPCR polypeptide according to the invention.

According to yet a further aspect of the present invention, there are provided cells or an animal genetically engineered to overexpress, underexpress or to exhibit targeted deletion of a GPCR polypeptide of the present invention.

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Detailed description of the invention

The present invention will now be described, by way of example only, with reference to 25 the accompanying figures and sequence listing, wherein:

Figure 1 shows the nucleotide sequence coding for PFI-021. The ATG translation initiation codon is indicated by the first three letters. The stop codon is indicated by the last three letters.

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Figure 2 shows the corresponding amino acid sequence coding for PFI-021.

Figure 3 shows a ClustalW Alignment of PFI-021 with Cysteinyl leukotriene receptor (CYSLT1).

SEQ ID NO: 1 is the nucleotide sequence coding for PFI-021. The ATG translation initiation codon is indicated by the first three letters. The stop codon is indicated by the last three letters.

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SEQ ID NO: 2 is the amino acid sequence for PFI-021.

SEQ ID NO: 3 is a ClustalW Alignment of PFI-021 with cysteinyl leukotriene receptor (CYSLT1).

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The polynucleotide which encodes the GPCR of the present invention was identified electronically and analysed using various bioinformatic tools. The GPCR encoded by the sequences described herein has been termed PFI-021.

- 15 The term "nucleotide sequence" as used herein refers to an oligonucleotide sequence or polynucleotide sequence, and variants, homologues, fragments and derivatives thereof (such as portions thereof). The nucleotide sequence may be DNA or RNA of genomic or synthetic or recombinant origin which may be double-stranded or single-stranded whether representing the sense or antisense strand. Preferably, the term "nucleotide sequence" 20 means DNA. More preferably, the term "nucleotide sequence" means DNA prepared by use of recombinant DNA techniques (i.e. recombinant DNA).

In a preferred embodiment, the present invention does not cover the native nucleotide coding sequence according to the present invention in its natural environment when it is 25 under the control of its native promoter which is also in its natural environment. For ease of reference, we shall call this preferred embodiment the "non-native nucleotide sequence".

- As used herein "amino acid sequence" refers to peptide or protein sequences or portions 30 thereof. The term "polypeptide", which is interchangeable with the term protein, refers to amino acid sequences, which are at least 5 amino acids in length, preferably at least 10 amino acids in length, more preferably at least 15 amino acids in length, yet more preferably at least 20 amino acids in length, preferably at least 50 amino acids in length.

In a preferred embodiment, the present invention does not cover the native PFI-021 polypeptide according to the present invention when it is in its natural environment and when it has been expressed by its native nucleotide coding sequence which is also in its natural environment and when that nucleotide sequence is under the control of its native 5 promoter which is also in its natural environment. For ease of reference, we shall call this preferred embodiment the "non-native amino acid sequence".

As used herein "biologically active" refers to a PFI-021 polypeptide having structural, regulatory or biochemical functions of the naturally occurring PFI-021. A polypeptide 10 according to the invention having "PFI-021 activity" has at least one of the structural, regulatory or biochemical functions of the native PFI-021 polypeptide. As used herein, "immunological activity" is defined as the capability of the natural, recombinant or synthetic PFI-021 peptide or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

15 The term "derivative" as used herein includes chemical modification of a PFI-021 polypeptide .

20 As used herein, the terms "isolated" and "purified" refer to molecules, either nucleic or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

The terms "variant", "homologue" or "fragment" in relation to the amino acid sequence 25 for the preferred polypeptide of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) amino acid from or to the sequence providing the resultant polypeptide has PFI-021 activity. In particular, the term "homologue" covers homology with respect to structure and/or function.

30 The terms "variant", "homologue" or "fragment" in relation to the nucleotide sequence coding for the preferred polypeptide of the present invention include any substitution of, variation of, modification of, replacement of, deletion of or addition of one (or more) nucleic acid from or to the sequence providing the resultant nucleotide sequence codes for or is capable of coding for a polypeptide having PFI-021 activity. In particular, the term

- "homologue" covers homology with respect to structure and/or function providing the resultant nucleotide sequence codes for or is capable of coding for an enzyme having PFI-021 activity. With respect to sequence homology (i.e. identity), preferably there is at least 70%, preferably at least 70-75%, more preferably at least 75%, preferably at least 75-
5 80%, more preferably at least 80%, preferably at least 80-85%, more preferably at least 85%, preferably at least 85-90%, more preferably at least 90%, preferably at least 90-95%, yet more preferably greater than 95% identity, preferably at least 98% identity to the polynucleotide sequence shown in Figure 1 and SEQ ID NO: 1.
- 10 In particular, the term "homology" as used herein may be equated with the term "identity". Relative sequence homology (i.e. sequence identity) can be determined by commercially available computer programs that can calculate percentage (%) homology between two or more sequences. A typical example of such a computer program is CLUSTAL.
15 As used herein, the terms "variant", "homologue", "fragment" and "derivative" include allelic variations of the sequences.
20 In relation to nucleic acids, the term "variant" also encompasses sequences that are complementary to sequences that are capable of hybridising to the nucleotide sequences presented herein. Preferably, the term "variant" encompasses sequences that are complementary to sequences that are capable of hybridising, when tested under stringent conditions (e.g. 65°C and 0.1xSSC {1xSSC = 0.15 M NaCl, 0.015 Na₃ citrate pH 7.0}), to the nucleotide sequences presented herein.
25 The present invention also covers nucleotide sequences that can hybridise to the nucleotide sequences of the present invention (including complementary sequences of those presented herein). In a preferred aspect, the present invention covers nucleotide sequences that can hybridise to the nucleotide sequence of the present invention under 30 stringent conditions when tested using standard laboratory techniques well known in the art (e.g. 65°C and 0.1xSSC).

The term "vector" includes expression vectors and transformation vectors.

The term "expression vector" means a construct capable of in vivo or in vitro expression.

The term "transformation vector" means a construct capable of being transferred from one species to another.

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The identification of PFI-021

PFI-021 was identified in unfinished genomic sequence information (GenBank contig accession AC083865) which is being released by the Genome Sequencing Centers by 10 searching the sequences with known members of the G-protein coupled receptor (GPCR) family using the BLAST algorithm. In order to confirm that PFI-021 was a member of the GPCR family, a number of bioinformatics approaches were performed.

a) BLAST Search against Swissprot

15 PFI-021 was searched against Swissprot using the BLAST algorithm (Basic Local Alignment Search Tool (Altschul SF (1993) J.Mol. Evol. 36:290-300; Altschul, SF et al (1990) J. Mol. Biol. 215:403-410)) to identify the closest protein match. In this case the top hit was to: Cysteinyl leukotriene receptor (CYSLT1).

20 These results indicate that PFI-021 is a member of the GPCR family.

(b) ClustalW Alignment of PFI-021 with Cysteinyl leukotriene receptor (CYSLT1)

These results are shown in Figure 3.

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(c) BLAST search against a non-redundant human GPCR database

PFI-021 was searched against a non-redundant human GPCR database comprising mainly sequences from Genbank and Geneseq Patents databases in order to identify the class of agonist for this receptor. The top hits are shown below:

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Source	Name	E-value
GENBANK	Cysteinyl leukotriene receptor (CYSLT1) ...	4e-20
GENBANK	G protein-coupled receptor GPR34 [L:381]	1e-18
GENESEQP	G-protein coupled receptor GRIR-2 (gene...)	7e-18
5 SWISSPROT	G protein-coupled receptor GPR18 (frag...)	7e-18
Genbank	Cysteinyl Leukotriene receptor CysLT2 (P...)	8e-17
SWISSPROT	P2Y purinoceptor 5 (P2Y5) [L:344]	1e-16
SWISSPROT	G protein-coupled receptor KIAA0001. U...	7e-16
SWISSPROT	G protein-coupled receptor GPR7. [L:328]	1e-15

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(e value = statistical likelihood of the hit occurring by chance)

These results demonstrate that PFI-021 is most closely similar to cysteinyl leukotriene receptors and they suggest that PFI-021 encodes a novel GPCR whose ligand is likely to be a cysteinyl leukotriene.

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15 (d) **Tissue distribution of PFI-021**

Expressed sequence tags of the PFI-021 sequence are found mainly in cDNA libraries prepared from peripheral blood mononuclear cells; two ESTs have also been identified in
20 libraries prepared from breast mRNA.

It will be appreciated that the foregoing is provided by way of example only and modification of detail may be made without departing from the scope of the invention.